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# The Effects of Chilling on the Fecundity and Life Span of Mass-reared Parasitoids (Hymenoptera: Braconidae) of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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*Suppression of Mediterranean fruit fly (medfly),* Ceratitis capitata *(Wiedemann), populations may be achieved through the mass-rearing and augmentative aerial release of opiine braconid parasitoids. Typically, aerial release techniques require up to one hour of chilling of adult parasitoids at temperatures as low as 3.5°C prior to their dissemination. Such chilling potentially could affect the subsequent performance of the insects. Among three species of the genus* Diachasmimorpha longicaudata *(Ashmead),* tryoni *(Cameron), and* krausii *(Fullaway) there was little or no affect of chilling in the laboratory on female longevity, production of daughters, or offspring sex ratio. This is consistent with previous experiments that found chilling to have no discernable effect on the short-term mortality of* D. tryoni *or on its ability to take flight immediately after aerial release. While there was little effect of chilling on longevity and fecundity in a species from another opiine genus,* Fopius arisanus *(Sonan), exposure to low temperatures did result in a significantly more male-biased offspring sex ratio.*

**Keywords:** *medfly,* Diachasmimorpha*, augmentative biological control, aerial releases*

## INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is a pest of over 300 species of fruits and vegetables (Liquido *et al.*, 1990). In addition to crop losses, it is responsible for the establishment of quarantines that prevent or hinder the development of agricultural exports. There would be serious economic consequences should the medfly

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become established in the continental United States. A University of California study estimated a $1.1 billion annual impact on California's economy due to trade embargos, loss of jobs, increased pesticide use, and direct crop losses (Siebert & Prahdan, 1991). At this time, prophylactic treatments of the Los Angeles Basin alone cost $15.4 million annually (Siebert & Cooper, 1995), and the 1998-99 campaign to eradicate medfly from the Tampa, Florida area cost ~$24 million (Florida Dept of Agriculture, internal documents).

Medfly is widely distributed across the tropics and subtropics. It was established in Hawaii near the turn of the century, and has been periodically introduced, and subsequently eradicated, in California, Florida and Texas (e.g. Clark *et al*., 1996). At one time the medfly invaded Mexico, but it was eradicated by the early 1980s through the use of insecticide (Malathion)-bait sprays and Sterile Insect Technique (SIT) (Hendrichs *et al*., 1983). However, dense populations persist throughout Central America and much of South America, particularly in the vast plantations of coffee in the highlands of Guatemala.

The northward spread of the medfly into Mexico, and ultimately into the continental United States, has been prevented by an SIT/bait-spray barrier maintained along the Guatemalan/Mexican border by the international organization MOSCAMED (USA, Mexico and Guatemala). Recently, the barrier has become increasingly permeable. For example, in 1998-1999 there were several medfly outbreaks on the Mexican side of the border (MOSCAMED, Internal documents). Additional control methods may be necessary to reestablish an effective barrier and/or eradicate Central American medfly populations.

Augmentative biological control, rearing large numbers of parasitoids and then periodically releasing them at appropriate times and places, may be an effective means of suppressing medfly in Guatemala. There are at least three contexts for augmentative releases: (1) Parasitoid releases in combination with SIT can cause greater mortality than either technique used alone. In an experiment using sterile medflies and the braconid *Diachasmimorpha tryoni* (Cameron), pest suppression levels were estimated to be 4.7 × higher than they would have been had SIT been used alone (Wong *et al*., 1992). This synergy may be crucial when insecticides cannot be used and non-chemical controls are necessary to effect eradication (e.g. organic agricultural areas, urban and suburban areas, nature preserves). (2) There can be special circumstances where neither bait-sprays nor SIT are practical. For example, in Florida, Caribbean fruit fly, *Anastrepha suspensa* (Loew), free zones are threatened by large pest populations in adjacent urban and suburban areas (Sivinski *et al*., 1996). Suppression of these populations through indefinitely repeated bait-sprays is not possible, and citrus growers are concerned that large numbers of sterile flies in the monitoring traps could delay or threaten their shipments. Augmentative parasitoid releases suffer none of these drawbacks. (3) Mathematical models suggest that augmented parasitoids that remain focused on tephritid populations, even when pests are at very low densities, could eradicate their hosts (Knipling, 1995; Barclay, 1987).

Previous augmentative parasitoid releases against medfly in Hawaii, Mexico, and Guatemala have, in general, been encouraging (e.g. Wong *et al*., 1991, 1992; Sivinski *et al*., 2000; J. Cancino *et al*., pers. comm.). The majority of releases were made from the ground, either by allowing pupae to emerge in the field or through the liberation of adults. However, aerial release techniques must be perfected to make parasitoid applications practical in the rugged Guatemalan highlands. A series of releases of adult *D. tryoni* dropped in torn paper bags from an airplane over a Guatemalan coffee plantation resulted in up to 84% medfly parasitism (Sivinski *et al*., 2000). While these parasitism levels are as high or higher than those obtained from ground releases, they resulted from relatively high release rates, particularly when compared with the earlier ground releases of *D. tryoni* by Wong *et al*. (1991, 1992). There were several possible reasons for the comparatively low efficacy of the Guatemalan aerial releases. These include a less favorable environment for *D. tryoni* in Guatemala than in Hawaii and differences in medfly host plants and densities between the two sites. There were also potential technical difficulties that might have adversely affected the parasitoids and made them less able to survive and forage for hosts. These include the

fall from the airplane and their chilling prior to packaging. In a subsequent study, expulsion from an airplane did not affect either *D. tryoni*'s immediate survival or their ability to take flight (Sivinski *et al*., 2000). Here we examine the long term effects of chilling on fruit fly parasitoid life span and fecundity.

Because parasitoids other than *D. tryoni* may prove better suited to aerial release in Guatemala, several additional species, *Diachasmimorpha longicaudata* (Ashmead), *krausii* (Fullaway) and *Fopius arisanus* (Sonan), also were chilled, and their subsequent longevities and reproductive capacities recorded. All are Old World opiine braconids originally collected from *Bactrocera* spp., and all are endoparasitic koinobionts (Wharton, 1997). The *Diachasmimorpha* spp. lay their eggs in late-instar fruit fly larvae and complete development in the host pupae, while *F. arisanus* oviposits in host eggs and completes development in the pupae.

## METHODS

All of the parasitoids tested had been reared in the MOSCAMED/USDA-APHIS 'Aurora' facility in Guatemala City, Guatemala for a period of at least 2 years. The colonies were derived from previously cultured stocks obtained from the University of Hawaii (*D. krausii*), USDA-ARS-Hawaii (*F. arisanus*), and MOSCAMED (Metapa Rearing Facility, Chiapas, Mexico) (*D. tryoni* and *longicaudata*).

Adult parasitoids packaged into paper bags or loaded into a dispersal machine for aerial release are first immobilized in a cold chamber and held at 3.5-4.5ºC for up to 60 min (MOSCAMED, standard operating procedures; Sivinski *et al*., 2000). In order to estimate the effects of this chilling on the fecundity and life span of *D. tryoni*, *D. longicaudata*, *D. krausii* and *F. arisanus*, cohorts of adult parasitoids (50 males and 50 females of the *Diachasmimorpha* spp. and 100 males and 100 females of *F. arisanus*) including females that reached sexual maturity in the presence of males, but which had no oviposition experience (5-8 days old), were transferred into $30 \times 30 \times 30$ cm plexiglass cages. Five to 8 days is the age at which adults have been released in previous augmentations (Sivinski *et al*., 1996, 2000). Eight of these cages per *Diachasmimorpha* species were set up for each treatment (chilled and unchilled-control), and each cage included a container of water with a sponge wick, a block of honey agar, and honey on the top screening. Because of lower adult availability four cages of each treatment were used in the case of *F. arisanus*. The four or eight chill-treatment cages for each species were placed in a refrigerator whose temperature had previously reached 2ºC. as measured by an Oakton digital thermometer. At the time of cage loading, the temperature control was reset to the coldest setting and temperature readings were made every 15 min. Initially the temperature typically rose to ca. 10ºC, but after approximately 2 h fell to 3.5ºC. At that point, the control was set to maintain that temperature, and the caged insects were exposed to 3.5ºC for 60 min. Cages were then taken out of the refrigerator and brought to a climate controlled room where they were placed on a table in an alternating pattern with the eight control cages. This room was held at 26ºC and 65% relative humidity (RH) for the remainder of the experiment.

Over the following 16 days (32 for *F. arisanus*), the numbers and sexes of dead insects were counted and recorded every morning. After the count, ~750 irradiated third instar *C. capitata* larvae were sandwiched between organdy cloth held in a 9cm diameter embroidery hoop ('sting ring'; Ramadan *et al*., 1989). A sting ring was then left in each cage for three hours, the standard exposure time at the rearing facility. Hosts were exposed to parasitoids five days a week (Monday through Friday), when larvae from the medfly rearing facility (El Pino, Guatemala) were available. Thus, fecundity data was gathered from the 12 exposure days that occurred during the 16 days of the experiment. After the three hour exposure, the larvae from each sting ring were placed in a 250 cc plastic cup with sawdust as pupation media. All cups were taken into a separate room (full darkness, 24-25ºC, 70% RH) where they remained for 14 days. Afterwards, the pupae were sifted to remove the sawdust and

returned to their fabric covered cup. Parasitoids were allowed to emerge and die (no food or water was provided), and were then counted and sexed.

Treatment of *F. arisanus* was similar to the above except for the presentation of hosts. Eggs of *C. capitata* were presented to the parasitoids in depressions formed in slices of papaya fruit, *Carica papaya* L. (see details in Harris & Bautista, 1996). Because *F. arisanus* proved to be longer lived on average than the *Diachasmimorpha* spp., fecundity data were obtained from 14 of the first 33 experimental days.

Mortality and offspring data were used to calculate the longevity (lx = proportion of female cohort remaining on successive days), fecundity (mx = female offspring per female per day of host exposure), and the product of lx and mx (lxmx) of each replicate in both treatments (Carey, 1993). These familiar values are commonly used to determine population growth, but here are used simply to estimate the vigor of the experimental cohorts and to provide a convenient means of comparing chilled and control insects. The lxmx values in particular constitute a concise summary of the reproductive status of the parasitoid cohorts. Offspring sex ratios (male/female) were calculated from the summed daily numbers of male and female offspring in treatment and control cohorts. Statistical analysis was by Student's *t*-test (SAS Institute, 1989) and Wilcoxon nonparametric paired-sample *T*-test (sex ratio data; Zar, 1974).

## RESULTS

*Diachasmimorpha tryoni*: Chilling produced no adverse effects on either female longevity (lx) or fecundity (mx) (Figure 1). On three days the mortality of the control insects significantly and unexpectedly exceeded that of the treated cohort. On two occasions, late in the life of the cohorts, the mean lxmx values of the chilled cohorts were significantly greater than those of the control (Figure 5(a)). Mean offspring sex ratios produced by chilled and control cohorts were both male biased (mean[control] = 1.25, SE = 0.18; mean[chilled] = 1.23, SE = 0.12) and did not differ significantly ($T = 26.0$, $n = 12$, $P > 0.25$).

*Diachasmimorpha krausii*: As with *D. tryoni*, chilling had no adverse effect on survival or fecundity (Figure 2 and 5(b)). On one occasion, late in the life of the cohort, control parasitoids produced significantly more daughters. Mean offspring sex ratios produced by the chilled and control cohorts were both female biased (mean[control] = 0.81, SE = 0.06; mean[chilled] = 0.76, SE = 0.07) and did not differ significantly ($T = 24.0$, $n = 12$, $P > 0.25$).

*Diachasmimorpha longicaudata*: There were no significant daily differences between chilled and control cohorts in terms of survival, fecundity, or lxmx values (Figure 3 and 5(c)). Mean sex ratios of the offspring produced by the chilled and control cohorts were both male biased (mean[control] = 1.29, SE = 0.07; mean[chilled] = 1.25, SE = 0.09), and did not differ significantly ($T = 21.5$, $n = 12$, $P > 0.10$).

*Fopius arisanus*: There were no significant daily differences between chilled and control cohorts in terms of fecundity (mx) and lxmx values (Figures 4 and 5(d)). In one instance, day 22 of the experiment, survival within the control cohort was significantly higher than in the chilled cohort. Mean sex ratios of the offspring produced by chilled and control cohorts were both female biased (mean[chilled] = 0.96, SE = 0.17; mean[control] = 0.65, SE = 0.05), and differed significantly; chilled parasitoids consistently produced relatively more males than controls ($T = 6.5$, $n = 14$, $P < 0.01$).

## DISCUSSION

Our results suggest that the lower than predicted efficacy of the previous aerial release of *D. tryoni* (Sivinski *et al.*, 2000) was not due to chilling of adults prior to dispersal. Neither adult female survival, the production of daughters, nor the offspring sex ratios differed substantially between chilled and untreated cohorts of *D. tryoni* or any of the other *Diachasmimorpha* species. In *F. arisnaus* there was little meaningful difference in fecundity

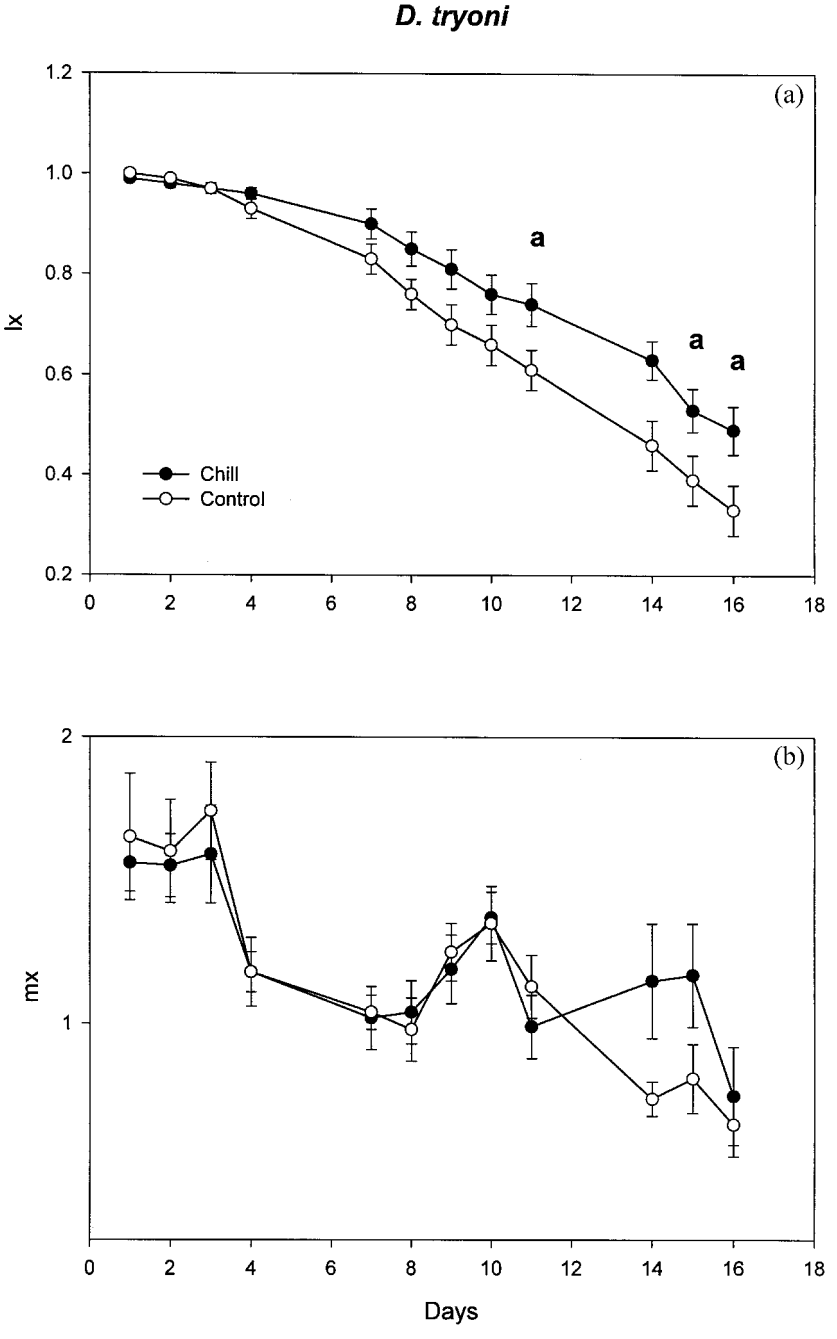


FIGURE 1. (a) The mean proportions and standard errors of the original cohorts of chilled and untreated *Diachasmimorpha tryoni* surviving on a daily basis (lx). Black circles refer to chilled parasitoids and open circles to controls. A small 'a' over a pair of means symbolizes a significant difference on that particular day. (b) The means and standard errors of the numbers of female offspring produced per adult female (mx) in chilled and control cohorts of *D. tryoni* over time. Symbols are as above.

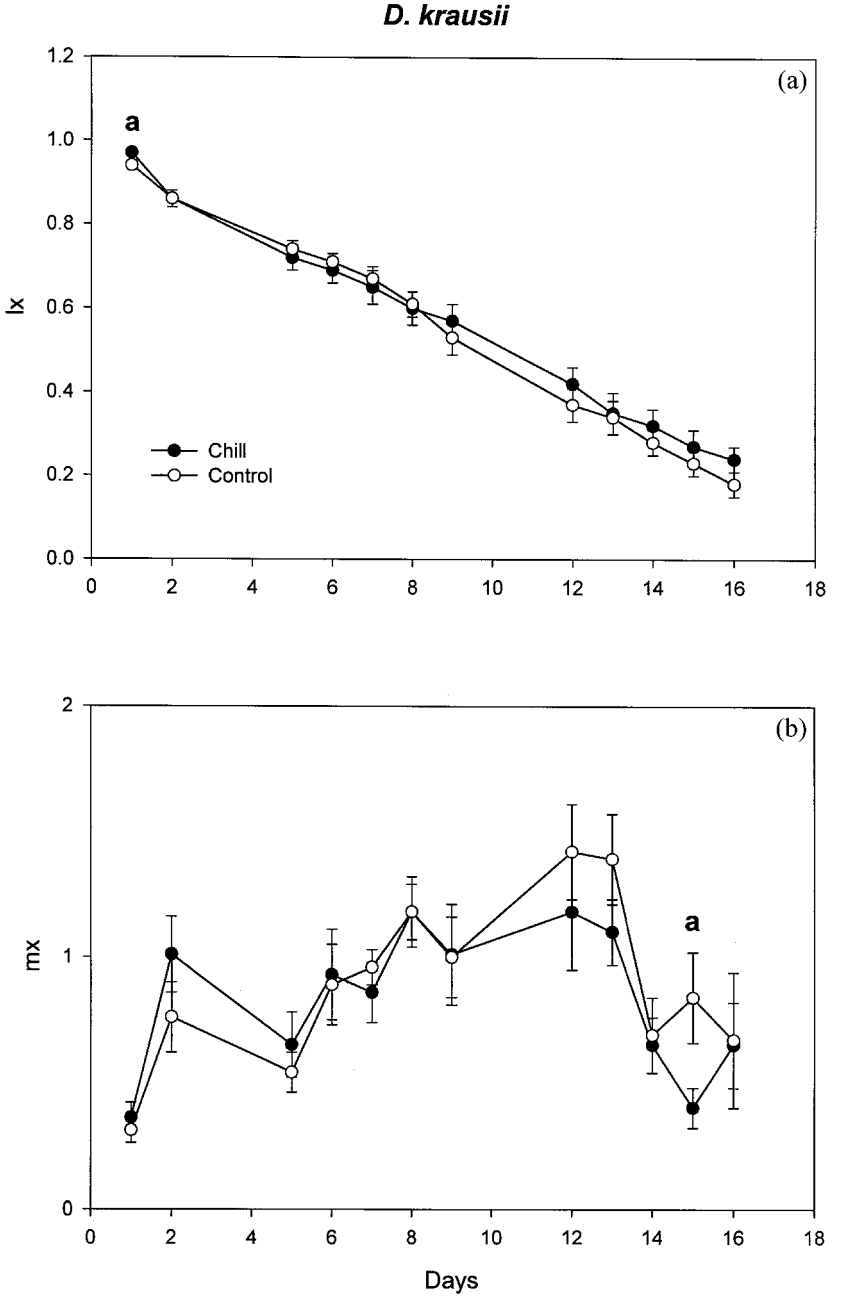


FIGURE 2. (a) The mean proportions and standard errors of the original cohorts of chilled and untreated *Diachasmimorpha krausii* surviving on a daily basis (lx). Black circles refer to chilled parasitoids and open circles to controls. A small 'a' over a pair of means symbolizes a significant difference on that particular day. (b) The means and standard errors of the numbers of female offspring produced per adult female (mx) in chilled and control cohorts of *D. krausii* over time. Symbols are as above.

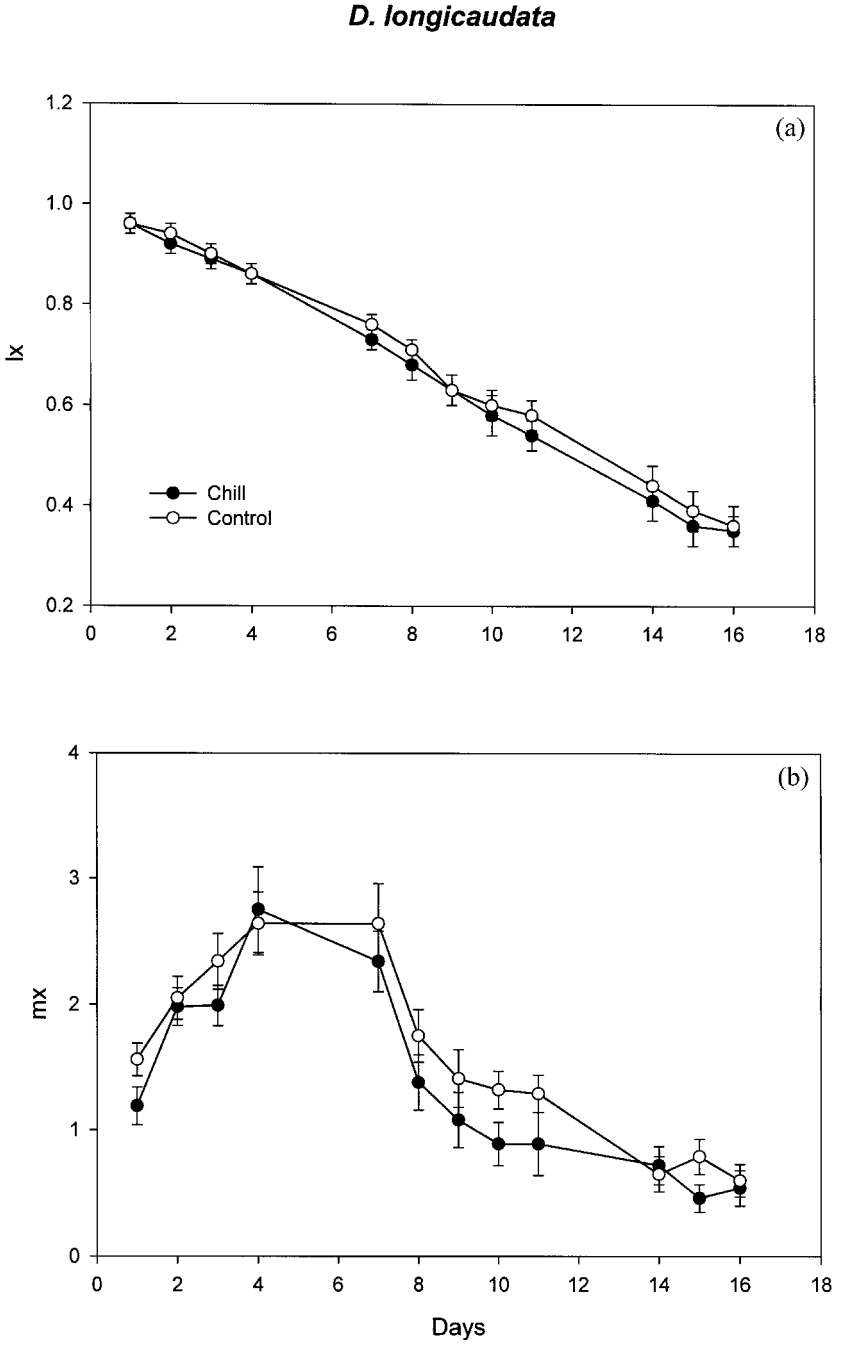


FIGURE 3. (a) The mean proportions and standard errors of the original cohorts of chilled and untreated *Diachasmimorpha longicaudata* surviving on a daily basis (lx). Black circles refer to chilled parasitoids and open circles to controls. A small 'a' over a pair of means symbolizes a significant difference on that particular day. (b) The means and standard errors of the numbers of female offspring produced per adult female (mx) in chilled and control cohorts of *D. longicaudata* over time. Symbols are as above.

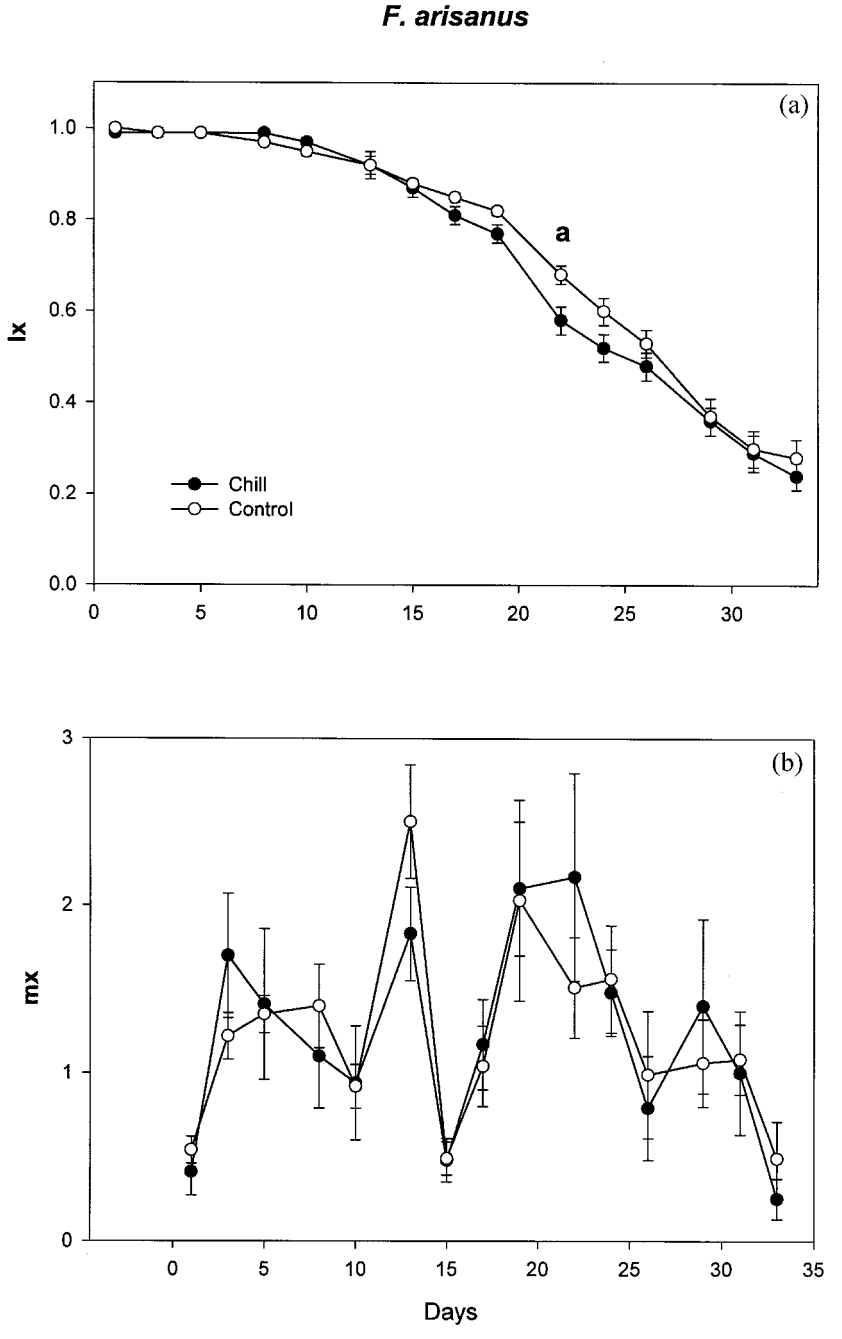


FIGURE 4. (a) The mean proportions and standard errors of the original cohorts of chilled and untreated *Fopius arisanus* surviving on a daily basis (lx). Black circles refer to chilled parasitoids and open circles to control. A small 'a' over a pair of means symbolizes a significant difference on that particular day. (b) The means and standard errors of the numbers of female offspring produced per adult female (mx) in chilled and control cohorts of *F. arisanus* over time. Symbols are as above.

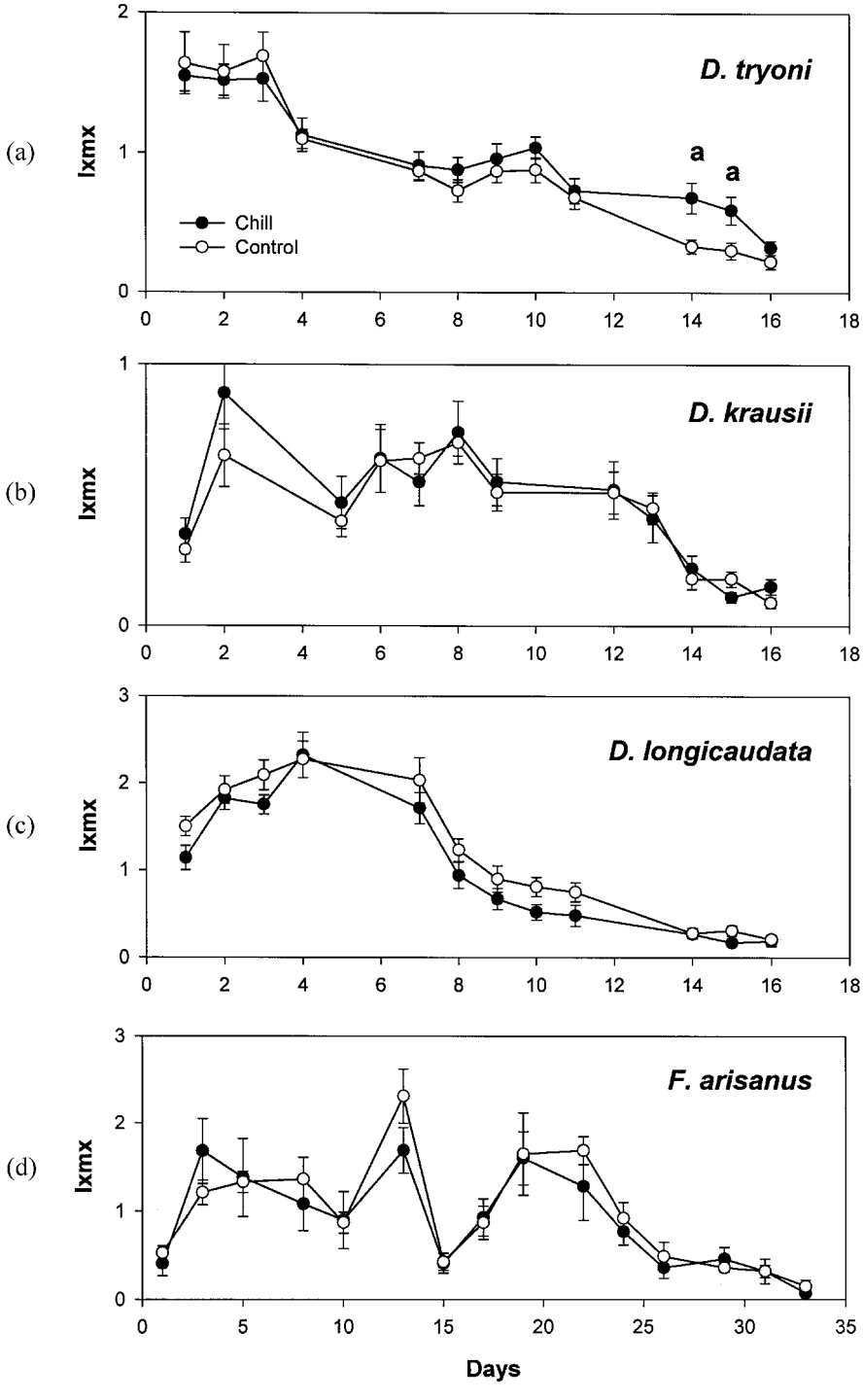


FIGURE 5. Means and standard errors of the products of lx and mx in chilled and untreated cohorts of: (a) *D. tryoni*; (b) *D. krausii*; (c) *D. longicaudata*; (d) *F. arisanus*.

or survival. However, there was a significant propensity for chilled females to produce relatively more male progeny. This general lack of long-term effects of chilling is consistent with the absence of noticeable effects on mortality and the propensity to take flight in *D. tryoni* immediately after aerial release (Sivinski *et al*., 2000).

Parasitoids were unaffected by chilling even though they were exposed to the lowest temperature (3.5°C) and for the longest time (1 h) called for by the MOSCAMED standard operating procedure. In actuality, insects in the laboratory were chilled even more extensively than those held in the large cooling unit used in the field. It took ~2 h for the laboratory insects to reach the final chilling temperature, while this was reached more quickly at the packaging site (~30 min) Thus, the laboratory insects were exposed to falling temperatures for a longer period of time, although cooling was experienced more gradually.

While the present data were gathered specifically to examine the effects of temperature, they might also be used to guide mass-rearing production and methods development. Under any given set of facility conditions and rearing procedures, some species may live longer and/or produce more offspring than others. A comparison of lxmx values would reveal which species, and which ages of a particular species, have the greatest potential for increase. If production efficiency were the sole criterion for choosing one of the examined parasitoid species for mass rearing, then *D. longicaudata* would be selected because it had the consistently highest lxmx values, particularly among females less than 13-16 days of age. On the other hand, a species may be chosen for mass-rearing because of its qualities in the field. Comparisons with lxmx values of congeners might indicate the need for rearing improvements. For example, preliminary field data showed *D. krausii* to be a promising candidate for augmentation (MOSCAMED, unpublished data), but its rearing clearly requires further development before it is as efficient as that of the closely related *D. longicaudata*.

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